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# *Anti-inflammatory, antipyretic and analgesic effect of Achillea millefolium and Salix plants*

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**The Anti-inflammatory, antipyretic and analgesic effect of *Achillea millefolium* and Salix were investigated in rats and mice. The extracts of *Achillea millefolium* exerted significant anti-inflammatory, antipyretic and analgesic effects. Also potentiated the sleeping time of thiopental sodium in mice. The doses used were 10.375 and 20.75 (watery extract) and 9.5 and 19 mg/100 gm b.wt. (ethanolic extract). Salix produced the same effects at doses of 0.0825 and 0.165 (watery extract) and 0.0850 and 0.170 mg/100 gm b.wt. (ethanolic extract). The extracts of both plants have an anti-inflammatory, antipyretic and analgesic effect.**

One of the most important fields of medicine is the herbal treatment. The back to nature invitation forced us to investigate plants used in folk medicine as *Achillea millefolium* and Salix. *Achillea millefolium* had been used in popular medicine for its anti-hemorrhagic, analgesic and wound healing effects (Chandler *et al*., 1982). It was used by Northern European and North American native peoples as a contraceptive, abortifacient and emmenagogue (Chandler *et al*., 1982). The flower heads of *Achillea millefolium* contain the most active fraction that posses anti-inflammatory activity (Goldberg *et al*., 1969). Extracts of the bark of Salix species had been used for treating fever, mild rheumatic complains and pain including headache. Salix contains active principle salicin, which is the pro-drug of various salicylate derivatives (Krivoy *et al*., 2001). The bark of Salix has anti-inflammatory properties (Schilcher, 2000).

The aim of this work was designed to investigate the effect of both water and ethanolic extracts of *Achillea millefolium* and Salix as anti-inflammatory, antipyretics and analgesics. The effect of extracts of both plants on sleeping time was also investigated.

## Materials and Methods

**Animals.** Ninety mature albino rats weighing 150-205 gm and hundred mice weighing 20-25 gm b.wt. of both sexes were obtained from Helwan Laboratory Animal Unit. Animals were kept and housed in plastic cages under hygienic conditions, fed on balanced ration and observed two weeks before use.

**Chemicals and drugs.** Ethanol 95 % (El-Nasr Pharmaceutical Chemical Co., Egypt), tween 80 solution (Fisher Chem. Alter Guide), dipyrone (Novalgin)® ampules (Hochest, Germany), paracetamol tablets (Pharco Co.), diclofenac sodium (Voltaren ampules)® (Ciba Gigey) and thiopental sodium (Nesdonal)® (Specia-Paris, France, MPH) were used in this study.

**Plant extracts.**

1-Acillea millefolium, the aerial parts of the plant was shade, dried and ground into fine powder and extracted. (a) Water extract was prepared by boiling 100 gm of dry powder with 300 ml distilled water for 10-15 min. Sieved and then the extract was evaporated until obtaining paste then dried. Solid extract was weighted and 10 gm were dissolved into 100 ml distilled water according to Chaplins'ka and Golovkin, (1962). (b) Ethanolic extract was prepared by putting 30 gm of dry powder in Soxlet apparatus with 95 % ethanol till obtain ethanolic extract then evaporate the extract until obtaining paste. Weigh the paste and dilute 10 gm with 100 ml tween 80 solution 1 % as a solvent.

2-The bark of Salix trees at least 4 years old was shade, dried and ground into fine powder and extracted as *Acillea millefolium*.

**Anti-inflammatory effect.** The method described by Winter *et al*. (1962) was adopted. Forty mature albino rats of both sexes weighing 150-200 gm were used. They were divided into 10 equal groups (4 rats in each). Edema in rats paw was induced by injecting 0.1 ml of Brewer's yeast 20 % suspension in physiological saline in paw skin (Randall and Sellitto**,** 1957). After four

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hours the thickness of the rat paw was measured using skin caliber to detect the inflammation achieved by the Brewer's yeast. The 1st group was left as control , while the 2nd group was given intraperitoneally diclofenac sodium (Voltaren)® ampoules in doses of 3.3 mg/100 gm b.wt. as an anti-inflammatory agent. The 3rd, 4th, 5th and 6th groups of Acillea millefolium and Salix were injected intraperitoneally with water extract in doses of 10.375 and 20.75, 0.0825 and 0.165 mg/100 gm b.wt., respectively. While the 7th, 8th, 9th and 10th groups were injected intraperitoneally with ethanolic extract at doses of 9.5 and 19, 0.0850 and 0.170 mg/100 gm b.wt., respectively. The thickness of the paw was measured after 3 and 6 hours after administration. The thickness of the paw measured before administration of Brewer's yeast was subtracted from the value obtained in order to estimate the edematous swelling. The activity of the drug was estimated from the percent of decrease in the paw thickness compared with control group.

**Antipyretic effect.** The antipyretic effect of Acillea millefolium and Salix (watery and ethanolic extracts) on the rats of feverish body temperature was determined using the method described by (Alperman,1972). Fifty fasting mature albino rats weighing 200-250gm b.wt. of both sexes were divided into 10 groups (5 rats in each). Four groups were used for each plant extract. Hyperthermia was induced by subcutaneous injection of 20 % Brewer's yeast suspension in physiological saline in the paws (Teotino *et al*., 1963) in a dose of 0.15 gm/100 gm b.wt. After 17 hours, the body temperature of each rat was recorded rectally using medical thermometer. The 1st group served as a control, injected with saline. The 2nd group was injected intraperitoneally with dipyrone (Novalgin)® ampoules in a dose of 5 mg/100 gm b.wt. as a standard antipyretic. The 3rd, 4th, 5th and 6th groups were injected with water extract of *Acillea millefolium* and Salix in doses of 10.375 and 20.75, 0.0825 and 0.165 mg/100 gm b.wt., respectively. While the 7th, 8th, 9th and 10th groups were injected with ethanolic extract at doses of 9.5 and 19, 0.0850 and 0.170 mg/100 gm b.wt. intraperitoneally of Acillea millefolium and Salix, respectively. The body temperature of each rat was recorded every half-hour for four hours.

**Analgesic effect.**The analgesic effect of *Acillea millefolium* and Salix ( watery and ethanolic extracts) were determined using the hot plate method described by (Janssen and Jageneau, 1957) and modified by (Jacob and Bosovski, 1961). Fifty mature mice of both sexes weighing 20-25 gm were divided into 10 groups (5 mice in each). Four groups were used for each plant extract. The 1st group left as control, while the 2nd group was given orally paracetamol tablets in a dose of 50 mg/100 gm b.wt. as an analgesic. The 3rd, 4th, 5th and 6th groups of *Acillea millefolium* and Salix were given water extract in doses of 10.375 and 20.75, 0.0825 and 0.165 mg/100 gm b.wt., respectively. While the 7th, 8th, 9th and 10th groups were injected with ethanolic extract at doses of 9.5 and 19, 0.0850 and 0.170 mg/100 gm b.wt., respectively. After 5 minutes each mouse was placed in beaker of 2 liters capacity immersed in water bath at 56°C controlled thermostatically. The time elapsed until the mouse licks its paw or jumps was considered as the reaction time and recorded as a measure of analgesic activity. Reaction time was recorded after 10, 20, 30, 60, 90 and 120 min. post-treatment. The reaction time was calculated and used as response to the given dose at the respective time interval.

**Effect on sleeping time.** The method described by Alperman, (1972) was adopted. Fifty mature mice of both sexes weighing 20-25 gm.b.wt. were divided into 10 equal groups (5 mice in each). Five groups were used for each plant. All animals were injected with thiopental sodium (Nesdonal)® 10 mg/100 gm b.wt. in saline solution 2.5 % intra-peritoneally. After 60 minutes, two groups (1st group in each plant) were lifted as control (injected with saline solution intra-peritoneally), while the 3rd, 4th, 5th and 6th groups of *Acillea millefolium* and Salix were injected with water extract in doses of 10.375 and 20.75, 0.0825 and 0.165 mg/100 gm b.wt., respectively. The 7th, 8th, 9th and 10th groups were injected with ethanolic extract at doses of 9.5 and 19, 0.0850 and 0.170 mg/100 gm b.wt., respectively. Time from loosing till remaining of the righting reflexes was considered as the sleeping time.

The obtained results were statistically analyzed using student "t" test according to Snedecor, (1969) and were expressed as mean and standard error (SE).

## Results

The antipyretic and analgesic effects of extracts of both *Achillea millefolium* (watery 10.375 and 20.75 and ethanolic 9.5 and 19 mg/100gm b.wt.) and Salix (watery 0.0825 and 0.165 and ethanolic 0.0850 and 0.170 mg/100 gm b.wt.)

**Table (1): Mean (±S.E) anti-inflammatory effect of *Achillea millefolium* and Salix extracts given I/P to rats (n=4).**

| Extract | Plant | Group | Dose (mg/100 gm b.wt) | Thickness of paw (mm) before Brewer's yeast injection | Initial | Thickness of paw (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 3h | % | 6h | % |
| Control | | 1st | - | 2.16±0.05 | 7.62±0.07 | 7.2±0.04 | 5.4 | 6.98±0.05 | 8.4 |
| Diclofenac sod. | | 2nd | 3.3 | 2.25±0.05 | 7.77±0.06 | 5.8±0.06** | 25.8 | 3.92±0.10 | 49.5 |
| Water | Achillea | 3rd | 10.375 | 2.15±0.06 | 7.02±0.10** | 5.8±0.05** | 18.1 | 5.32±0.02** | 24.2 |
| | | 4th | 20.750 | 2.15±0.06 | 7.07±0.07** | 5.1±0.04** | 27.4 | 4.8±0.07** | 31.6 |
| | Salix | 5th | 0.0825 | 2.4±0.04 | 7.5±0.04 | 6.8±0.03** | 18.8 | 6.1±0.04** | 24.2 |
| | | 6th | 0.165 | 2.3±0.10 | 7.4±0.06 | 5.9±0.10** | 28.4 | 5.4±0.09** | 34.7 |
| Ethanolic | Achillea | 7th | 9.5 | 2.19±0.03 | 7.02±0.10** | 5.7±0.06** | 9.3 | 5.32±0.04** | 18.6 |
| | | 8th | 19 | 2.15±0.03 | 7.07±0.10** | 5.02±0.09** | 20.1 | 4.52±0.10** | 27.7 |
| | Salix | 9th | 0.0850 | 2.3±0.09 | 7.4±0.04* | 6.7±0.05** | 9.18 | 5.9±0.08** | 19.5 |
| | | 10th | 0.170 | 2.3±0.08 | 7.2±0.08 ** | 5.7±0.19** | 20.1 | 5.2±0.11** | 27.7 |

* p< 0.05 **p<0.01

were recorded in Tables (1) and (2), respectively. Both plantsin different extracts produced significant anti-inflammatory effect as reflected by decrease in paw thickness (Table 3). Both watery and ethanolic extracts of *Achillea millefolium* and Salix produced significant (p<0.01) increase in the sleeping time of thiopental sodium in mice when compared with control (Table 4).

## Discussion

Both watery and ethanolic extracts of *Achillea millefolium* produced a significant decrease in body temperature in feverish rats at doses of 10.375, 20.750 and 9.5, 19 mg/100 gm b.wt., respectively. This indicated that the extracts have antipyretic effect. These results are in agreement with those obtained by Kudrica and Glowniak (1967). They were attributed the antipyretic action of *Achillea millefolium* to its flavonoids. Moreover, this result similar to that obtained by Tierra and Lesley (1992). They were stated that *Achillea millefolium* has antipyretic effect.

Both watery and ethanolic extracts of Salix induced an antipyretic effect which recorded at doses of 0.0825, 0.160 and 0.0850, 0.170 mg/100 gm b.wt., respectively. These results agreed with that obtained by Kolodziej (1990), who reported that isolation and characterization of four dimeric and five trimeric procyanidins from Salix bark claimed to have antipyretic effect. Also similar effects approved by Thapliyal and Bahuguna (1993) and Masika *et al*. (1997). The antipyretic effect interpreted by Krivoy *et al*. (2001), they reported that white yellow bark (Salix bark) contain a variety of chemical constituents, the main one studied being salicin which is metabolically converted in the body to the aspirin metabolite, salicylic acid. Salicylates have antipyretic (fever lowering) effect.

*Achillea millefolium* (watery and ethanolic extracts) induced a significant analgesic effects at doses used. These findings agreed with Petcu and Anderonescu (1978) and Tierra and Lesley (1992). The analgesic effect of the extract explained by Levine (1978) who mentioned that the analgesic effect may be due to inhibition of synthesis of prostaglandin and as a result prevent the sensitivity of pain receptors.

Analgesic effect of watery and ethanolic extracts of Salix was recorded at doses of 0.0825, 0.165 and 0.0850, 0.170 mg/100gm b.wt., respectively. These results are in accordance with those obtained by Whang *et al*. (1995). They were stated that the bark of Salix gilgiana used as an analgesic. They mentioned that this action is may be due to dimeric and trimeric procyanidins. Also, Kolodziej (1990) showed that isolation and characterization of four dimeric and five trimeric procyanidins from Salix bark claimed to have analgesic effect. Furthermore, Eisenberg *et al*. (2000) and Heide *et al*. (2000) mentioned that Salix bark extracts produced analgesic activity in patients with osteoarthritis and effective in alleviating low

**Table (2): Mean (±S.E) antipyretic effect of *Achillea millefolium* and Salix extracts given I/P to hyperthermic rats (n=5).**

| Extract | Plant | Group | Dose (mg/ 100 g. b.wt) | Temperature before Bewer's yeast injection | The initial temperature | Rectal temperature C° (Mean±S.E.) after (time/min.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 |
| **Control** | | **1st** | **-** | 37.11 ±0.1 | 38.21 ±0.13 | 38.11 ±0.13 | 38.03 ±0.18 | 38.14 ±0.15 | 38.17 ±0.12 | 38.16 ±0.03 | 38.13 ±0.14 | 38.15 ±0.17 | 38.14 ±0.16 |
| **Dipyrone** | | **2nd** | **5** | 37.4 ±0.07 | 38.96 ±0.04 | 38.32 ±0.05 | 37.98 ±0.081 | 37.5** ±0.32 | 37.42** ±0.038 | 37.26** ±0.24 | 37.22** ±0.020 | 37.16** ±0.024 | 37.12* ±0.38 |
| **Water** | **Achillea** | **3rd** | **10.375** | 37.44 ±0.04 | 39.3 ±0.05 | 39.2 ±0.03 | 39.1 ±0.031 | 39.02 ±0.021 | 38.84 ±0.024 | 38.12 ±0.124 | 38 ±0.089 | 37.5* ±0.10 | 37.5** ±0.07 |
| | | **4th** | **20.750** | 37.48 ±0.02 | 39.3 ±0.09 | 39.2 ±0.07 | 39.04 ±0.67 | 38.86 ±0.06 | 38.74 ±0.067 | 38 ±0.063 | 37.7* ±0.07 | 37.5** ±0.031 | 37.5** ±0.07 |
| | **Salix** | **5th** | **0.0825** | 37.52 ±0.02 | 39.4 ±0.09 | 39.12 ±0.05 | 38.96 ±0.050 | 38.82 ±0.048 | 38.58 ±0.037 | 38 ±0.07 | 37.78 ±0.08 | 37.5** ±0.063 | 37.42** ±0.066 |
| | | **6th** | **0.165** | 37.44 ±0.04 | 39.2 ±0.06 | 38.96 ±0.05 | 38.8 ±0.044 | 38.54 ±0.04 | 38.3 ±0.054 | 37.8** ±0.089 | 37.6* ±0.089 | 37.5** ±0.044 | 37.3** ±0.063 |
| **Ethanolic** | **Achillea** | **7th** | **9.5** | 37.5 ±0.03 | 39.4 ±0.04 | 39.2 ±0.03 | 39.02 ±0.02 | 38.82 ±0.02 | 38.7 ±0.031 | 38.6 ±0.031 | 38.3 ±0.07 | 37.6* ±0.054 | 37.5 ±0.089 |
| | | **8th** | **19** | 37.5 ±0.03 | 39.4 ±0.04 | 39.34 ±0.05 | 39.14 ±0.05 | 38.92 ±0.058 | 38.64 ±0.092 | 38.2 ±0.083 | 37.8 ±0.089 | 37.5** ±0.054 | 37.5** ±0.054 |
| | **Salix** | **9th** | **0.0850** | 37.52 ±0.02 | 39.4 ±0.04 | 39.18 ±0.03 | 39.02 ±0.020 | 38.8 ±0.054 | 38.6 ±0.044 | 38.2 ±0.083 | 37.8 ±0.054 | 37.6* ±0.031 | 37.4** ±0.044 |
| | | **10th** | **0.170** | 37.48 ±0.02 | 39.3 ±0.06 | 39.1 ±0.09 | 38.82 ±0.058 | 38.6 ±0.063 | 38.2 ±0.07 | 38 ±0.031 | 37.6** ±0.044 | 37.42** ±0.058 | 37.4** ±0.054 |

* $p < 0.05$ ** $p < 0.01$

**Table (3): Mean (± S.E) analgesic effect of *Achillea millefolium* and Salix extracts given I/P to mice using hot plate method (n=5).**

| Extract | Plant | Group | Dose (mg/100 gm b.wt) | Reaction time (minutes) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 10 | 20 | 30 | 60 | 90 | 120 |
| Water | Control | 1st | - | 7.29±0.01 | 7.65±0.02 | 7.81±0.02 | 7.86±0.023 | 7.87±0.01 | 7.87±0.014 |
| | Paracetamol | 2nd | 50 | 7.47±0.02** | 8.35±0.02** | 9.57±0.01** | 10.91±0.010** | 12.52±0.04** | 14.35±0.020** |
| | Achillea | 3rd | 10.375 | 7.5±0.05* | 7.8±0.02** | 7.8±0.02 | 8.0±0.040* | 8.1±0.04 | 8.25±0.104** |
| | | 4th | 20.750 | 7.8±0.04** | 7.92±0.02** | 8.02±0.06* | 8.15±0.028** | 8.2±0.04** | 8.30±0.040** |
| | Salix | 5th | 0.0825 | 7.47±0.04** | 7.8±0.01 | 7.8±0.08 | 10.02±0.085** | 8.1±0.05 | 13.7±0.070** |
| | | 6th | 0.165 | 7.77±0.06** | 7.9±0.1 | 8.22±0.13* | 10.55±0.086** | 8.52±0.07** | 14.2±0.070** |
| Ethanolic | Achillea | 7th | 9.5 | 7.2±0.08 | 8.05±0.06** | 8.9±0.05** | 7.91±0.042 | 11.62±0.07** | 8.27±0.025** |
| | | 8th | 19 | 7.8±0.07** | 8.5±0.2** | 9.27±0.10** | 8.4±0.091 | 12.32±0.13** | 8.62±0.075** |
| | Salix | 9th | 0.0850 | 7.87±0.04** | 8.25±0.05** | 9.77±0.02** | 10.5±0.40** | 11.77±0.06** | 13.9±0.057** |
| | | 10th | 0.170 | 7.72±0.04** | 8.67±0.07** | 9.9±0.07** | 11.1±0.057 | 12.85±0.02** | 14.42±0.047** |

* p< 0.05 **p<0.01

**Table (4): Effect of *Achillea millefolium* and Salix extracts on sleeping time of thiopental sodium anaesthetized mice (n=5).**

| Extract | Plant | Group | Dose (mg/100 gm b.wt) | Sleeping time (hour) |
|---|---|---|---|---|
| Control | Achillea | 1st | - | 9.18±0.029 |
| | Salix | 2nd | - | 9.41±0.019 |
| Water | Achillea | 3rd | 10.375 | 9.41±0.055** |
| | | 4th | 20.750 | 9.99±0.033** |
| | Salix | 5th | 0.0825 | 9.98±0.010** |
| | | 6th | 0.165 | 11.0±0.225** |
| Ethanolic | Achillea | 7th | 9.5 | 9.32±0.058** |
| | | 8th | 19 | 9.52±0.012** |
| | Salix | 9th | 0.0850 | 10.51±0.012** |
| | | 10th | 0.170 | 11.22±0.012** |

**p<0.01

back pain. *Achillea millefolium* (watery and ethanolic extracts) produced a significant increase in sleeping time at doses of 10.375, 20.750 and 9.5, 19 mg/100 gm b.wt., respectively. The central nervous system depressant activity of *Achillea millefolium* may be attributed to the volatile oil present in the extracts (Christine, 2003).

Both watery and ethanolic extracts of *Achillea millefolium* and Salix posses an anti-inflammatory effect as indicated by decreasing thickness of paw in rats post-administration. The most active fractions isolated from *Achillea millefolium* flower heads posses anti-inflammatory activity that reduced the inflammation in mice by 35 % (Goldberg *et al.,* 1969). Most of species are widely used as anti-inflammatory and this effect may be due to the flavonoids present (Valant-Vetscheram, and Wollenweber 1988).

The anti-inflammatory effect of Salix explained by (Masika *et al*., 1997; Schilcher, 2000 and Krivoy *et al*., 2001). They were mentioned that white willow bark (Salix alba L) contains a variety of chemical constituents. The main one studied being salicin which is metabolically converted in the body to the aspirin metabolite, salicylic acid. Salicylates have anti-inflammatory effect in the body.

From the aforementioned results, watery and ethanolic extracts of both *Achillea millefolium* and Salix could be used as an anti-inflammatory, antipyretic and analgesic.

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## تأثير نباتي الأخيليا (ذات الألف ورقة) والصفصاف كمضاد للالتهاب ، خافض للحرارة ومسكن للألم

تم في هذا البحث دراسة تأثير خلاصة نباتي الأخيليا (ذات الألف ورقة) والصفصاف المائية والكحولية كمضادة للالتهابات ، خافضه للحرارة و مسكنه للألم وكذلك التأثير على ساعات النوم كمخدرة. وقد أظهرت الدراسة أن الخلاصة المائية والكحولية لنباتي الأخيليا والصفصاف قد أحدثتا انخفاضا معنويا في درجة حرارة جسم الفئران المحمومة وأثر معنوي مسكن للألم عند الجرعات ١٠,٣٧٥، ٢٠,٧٥٠، ٩,٥، ١٩، ٠,٠٨٢٥، ٠,١٦٥، ٠,٠٨٥٠ و ٠,١٧٠ مجم/١٠٠ جم من وزن الجسم على الترتيب. كما أحدثت نفس الجرعات السابقة لخلاصة النباتين المائية والكحولية نقصا معنويا في أثر الالتهاب بنسبة ١٨,٠٩ ، ٢٧,٣٥ و ٩,٣ ، ٢٠,٠٨ وكذلك ١٨,٨ ، ٢٨,٤ و ٩,١٨ ، ٢٠,٠٨ % بعد ٣ ساعات من الحقن وبعد ٦ ساعات من الحقن قل أثر الالتهاب بنسبة ٢٤,٢١ ، ٣١,٦ و١٨,٦ ، ٢٧,٧ وكذلك ٢٤,٢١ ، ٣٤,٧٢ و ١٩,٥ ، ٢٧,٧ % لكل من الخلاصتين على الترتيب. كما سجلت الدراسة وجود زيادة معنوية في ساعات النوم عند الفئران التي تم حقنها بالخلاصة المائية والكحولية لنباتي الأخيليا والصفصاف. تبين من هذه الدراسة أنه يمكن استخدام خلاصة نباتي الأخيليا والصفصاف المائية والكحولية كمضادة للالتهابات ، خافضة للحرارة و مسكنة للألم.